Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2006-10

Abietiv
Neodiprion abietis Nucleopolyhedrovirus Newfoundland Strain

The technical grade active ingredient Abietiv Technical and its end-use product Abietiv, containing the active ingredient *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV) Newfoundland strain, are proposed for temporary registration under the Pest Control Products Regulations for use as an insecticide to suppress balsam fir sawfly (*Neodiprion abietis*) in forest stands.

This Regulatory Note provides a summary of data reviewed and the rationale for the regulatory decision concerning these biopesticide products.

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Foreword

Pursuant to the Pest Control Products Regulations, Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registrations for the technical grade active ingredient Abietiv Technical, and its end-use product, Abietiv, developed by the Canadian Forest Service, to reduce populations of balsam fir sawfly (*Neodiprion abietis*) in forest stands.

Microbial pest control agents are increasingly being investigated for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment, compared with conventional pesticides. Abietiv represents a potential biological replacement for chemical insecticides.

This Regulatory Note presents a summary of the PMRA's findings in support of this decision.

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1.0 The Active Substance, Its Properties and Uses

1.1 Identity of the Active Substance and Impurities

Microbial Pest Control Agent Identification

Active microorganism	Polyhedral inclusion bodies (PIBs) of balsam fir sawfly nucleopolyhedrovirus (NPV) Newfoundland strain
Function	Viral insecticide
Binomial name	PIBs of *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV)
Taxonomic designation	
Family	Baculoviridae
Genus	*Nucleopolyhedrovirus*
Canadian Patent Status Information:	N/A
Nominal purity of active	4.0×10^9 PIBs/mL
Identity of relevant impurities of toxicological, environmental and/or other significance	The technical grade Abietiv Technical does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards, and no mammalian toxins are known to be produced by the technical grade active ingredient, Abietiv Technical.

1.2 Physical and Chemical Properties of Active Substances and End-Use Product(s)

Technical Product—Abietiv Technical

Property	Result
Colour	Brown
Odour	Musty
Physical state	Suspension
Specific gravity	1.00 g/mL
Viscosity	1.0 centipoise

Property	Result
Corrosion character (oxidizing or reducing action)	Not corrosive

End-Use Product—Abietiv

Property	Result
Colour	Brown
Odour	Musty
Physical state	Suspension
Formulation type	Suspension
Guarantee	4.0×10^9 PIBs/mL
Formulants	The product does not contain any United States Environmental Protection Agency List 1 formulants or formulants known to be TSMP Track 1 substances.
Container material and description	High density polyethylene (40 mL–20 L)
Corrosion character (oxidizing or reducing action)	Not corrosive
Density/bulk density/specific gravity	1.0 g/mL
pH (in solution)	Neutral
Storage stability	Information on storage stability indicated that Abietiv was stable at 4°C for up to 5 months

1.3 Details of Uses and Further Information

NeabNPV has been reported in populations of balsam fir sawfly from Alberta, Saskatchewan, Manitoba, Ontario, Quebec and Newfoundland and Labrador. The strain of NeabNPV used in the manufacture of Abietiv products was isolated from balsam fir sawfly larvae collected near Corner Brook, Newfoundland (Ecozone 5). Amplification of the virus was carried out by applying the virus in-field to balsam fir foliage and rearing larvae on the treated foliage.

Abietiv is an end-use product containing the active ingredient NeabNPV Newfoundland strain. Abietiv is proposed for use as a biological insecticide to reduce populations of balsam fir sawfly (*Neodiprion abietis*) on forest stands and woodlots (Use-site Category # 4). Abietiv is to be applied in an aqueous solution prepared by diluting 40–120 mL of product in 400 L of 20% aqueous molasses. The diluted solution is applied via aerial spraying at a rate of 2.5 L/ha

(1.0×10^9 to 3.0×10^9 PIBs/ha). Applications should be timed to coincide with sufficient foliage development and, when possible, after egg hatch has been completed.

The target organism is the Balsam fir sawfly (*Neodiprion abietis*), a member of the order hymenoptera. Larval infection begins with ingestion of the polyhedral inclusion bodies (PIBs) associated with NeabNPV. In the alkaline environment of the larval midgut, the protein coating the PIBs is dissolved to release the virions that infect the midgut epithelial cells where viral replication occurs. At late stages of infection, virions are occluded in the nucleus by polyhedrin protein to form PIBs. Infected cells, containing PIBs, are sloughed off into the frass and out of the body where they can be ingested and subsequently infect other host insects. Host death normally occurs within one to two weeks in which time host feeding is reduced or completely suppressed (Garcia-Maruniak et al. 2004).

Most baculovirus species infect only one or a few closely related invertebrate species (Kool et al. 1995). The host range of hymenopteran NPVs are reportedly narrower than the lepidopteran NPVs (Cunningham and Entiwistle 1981, Ignoffo and Couch 1981). NeabNPV is known to only infect and replicate in the midgut cells of balsam fir sawfly larvae.

2.0 Methods of Analysis

2.1 Methods for Analysis of the Microorganism as Manufactured

2.1.1 Methods for Identification of the Microorganism

Restriction endonuclease digests have been used to distinguish between NeabNPV isolated from Newfoundland (Abietiv) and NeabNPV isolated from elsewhere, specifically Nova Scotia. In this procedure, NeabNPV DNA is isolated using DNAzol (Invitrogen Life Technologies). The isolated DNA is combined with one of four restriction endonucleases, EcoRI, Xho1, Xba1 and Sal1 and incubated at 37°C for 18 hours. The digests are then analyzed by gel electrophoresis and stained with ethidium bromide. Digestion with EcoRI, Xba1 and Sal1 generate banding patterns that could distinguish between the two NeabNPV isolates. This method will be used to check each production batch against the NeabNPV master stock.

Other potential methods for confirming preservation of the strain include DNA sequencing of NeabNPV Newfoundland strain and the use of polymerase chain reaction with specific primers for variable and highly conserved regions. These methods, however, are in the preliminary stages of development and are not currently in routine use. Detailed protocols and expected results will be required if these methods are pursued.

2.1.2 Methods for Establishment of Purity of Seed Stock

The original Newfoundland strain of NeabNPV has been stored frozen at -20°C in dead larval hosts (*Neodiprion abietis*). Larvae infected with NeabNPV Newfoundland strain have also been deposited with the National Tree Seed Centre (Natural Resources Canada, Canadian Forest

Service—Atlantic Forestry Centre) in Fredericton, New Brunswick, where they are stored in cryovials and frozen in liquid nitrogen.

The master stock of NeabNPV Newfoundland strain consists of NeabNPV-infected balsam fir sawfly larvae from the 2003 and 2004 production years. The master stock will be used for NeabNPV production batches. The master stock will be replenished with the production batch once they are shown to be identical using the restriction endonuclease digestion method outlined in Section 2.1.1.

Given the in-field manufacturing process required for Abietiv, all batches must be subjected to appropriate quality assurance protocols, including an analysis and characterization of the active ingredient (e.g., by restriction endonuclease digestion) to ensure consistency between production batches.

2.1.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

Abietiv is produced by applying NeabNPV Newfoundland strain to balsam fir trees infested with balsam fir sawfly larvae. Polyhedral inclusion bodies are purified from dead insects by a process that includes filtration and centrifugation steps.

A proportional counting method is used to quantify the active ingredient. According to the method, a known volume and known concentration of standard particles is mixed with a known volume and unknown concentration of the sample. In this case, the standard particles consist of latex beads and the sample is the NeabNPV preparation. The mixture is smeared onto a microscope slide, stained and examined under a microscope. The average ratio of latex beads to NeabNPV polyhedra is determined based on a number of replicates. The average ratio is then used to calculate the concentration of NeabNPV polyhedra in the sample. Potency data for five batches of Abietiv were submitted and ranged from $2.5 \times 10^9 - 6.54 \times 10^9$ PIBs/mL. Batches that fall below the 4×10^9 PIBs/mL guarantee are concentrated and those that are significantly above the target potency are diluted in order to meet the guarantee.

The potency of Abietiv is also expressed as the number of PIBs that provide a lethal dose to 50% of the test insects (LD_{50}). For 2nd instar larvae, the LD_{50} and LD_{95} values were found to be 12.2 PIBs/larva (95% confidence interval: 4.3–26.3 PIBs/larva) and 1.4×10^4 PIBs/larva (95% confidence interval: 8.0×10^3–2.8×10^4 PIBs/larva), respectively. For 3rd instar larvae, the LD_{50} and LD_{95} values were found to be 4.8×10^2 PIBs/larva (95% confidence interval: 1.7×10^2–1.1×10^3 PIBs/larva) and 6.4×10^4 PIBs/larva (95% confidence interval: 3.8×10^4–1.2×10^5 PIBs/larva), respectively.

2.1.4 Methods for the Determination of Relevant Impurities in the Manufactured Material

Sawfly NPVs must be produced in the field as there are no established insect cell lines or artificial diets available for laboratory-rearing of *N. abietis*. Field production, however, does

present the potential for contamination by microbes that are part of the microflora of the larvae and the environment and although the manufacturing process includes steps to purify the NeabNPV, cells, tissues, fats, proteins, nucleic acids and other contaminants derived from the insect host may remain in the product. *Bacillus thuringiensis* (Bt) may also appear as a contaminant as the aircraft used to apply NeabNPV is also used to spray Bt.

All batches of Abietiv are tested for microbial contaminants using recognized methods. The applicant has proposed the following limits for acceptance:

Test	Limit (colony forming units/mL)
Total aerobic bacteria	5.8×10^5
Total coliforms	$> 1.6 \times 10^5$
Aerobic sporeformers	3.6×10^4
*Bacillus cereus**	6.5×10^4
Molds	9.5×10^2
Yeast	1.0×10^2
Escherichia coli	Negative
Fecal coliforms	< 2
Fecal *Streptococcus/Enterococcus*	Negative
Salmonella sp.	Negative
Shigella sp.	Negative
Staphylococcus aureus	< 250
Vibrio sp.	Negative

Given the difficulty in distinguishing between *B. cereus* and *B. thuringiensis*, an additional microscopic analysis for spores and parasporal crystals characteristic of *B. thuringiensis* must be included and a combined limit should be proposed for *B. cereus* and *B. thuringiensis* should contamination occur. Once a contaminant is identified as *B. thuringiensis*, it is important to determine its origin (i.e., registered strain of *B. thuringiensis* applied using the same aerial application equipment that is used for field production of Abietiv or wild-type strain).

Other NPVs and insect viruses may also infect sawflies resulting in their co-purification with NeabNPV. Given the in-field production method and hence the lack of control over the insect population used in the manufacture of Abietiv, the potential for contaminating insect viruses exists. Based on the available literature, however, only NPVs of other closely related sawflies

infect balsam fir sawfly. Sawfly NPVs, unlike other NPVs, have a narrow host range limited to closely related species.

No mammalian viruses have been known to be isolated from field-collected sawflies. However, the mouse batch intraperitoneal assay (see Section 2.1.5) and microbial contaminant testing, which are required on every production lot of Abietiv, would be capable of detecting primary mammalian pathogens.

2.1.5 Methods to Show Absence of Any Human and Mammalian Pathogens

A mouse intraperitoneal analysis is done on every batch of Abietiv to ensure the absence of human and mammalian pathogens. According to the submitted protocols, a total of 5 CD-1 mice are each administered 1.0×10^6 to 3.9×10^6 PIBs/mL via a single interaperitoneal injection. The animals are observed for seven days following injection and their body weights are monitored.

2.1.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Storage stability data have not been generated although efficacy data, using five-month old formulations of Abietiv stored at 4°C, were conducted. As the stability of Abietiv cannot be adequately assessed based on the submitted information, a maximum storage period of five months at 4°C is supported on a temporary basis. Additional storage stability data will be required as a condition of temporary registration.

2.2 Methods to Determine and Quantify Residues (viable or non-viable) of the Active Microorganism and Relevant Metabolites

Abietiv is a restricted class product for application to forests. The product is not intended for direct use on food or feed crops. Therefore, methods to quantify NeabNPV Newfoundland strain residues in food and feed are not required. Moreover, results of human health and safety tests indicated low acute oral toxicity, and product characterization information indicated a low potential for mammalian toxin production. Thus, NeabNPV Newfoundland strain does not satisfy the criteria for requiring the establishment of a maximum residue limit (MRL).

3.0 Impact on Human and Animal Health

See Table I of Appendix I, for Toxicology summary table.

3.1 Integrated Toxicity and Infectivity Summary

The information and data submitted by the Canadian Forest Service, in support of registration of Abietiv Technical and Abietiv, along with published literature on related baculoviruses, were reviewed from the viewpoint of human health protection and were determined to be sufficiently complete to permit a decision on registration.

For the most part, the information provided by the applicant on the characterization of the active ingredient as well as the manufacturing process and quality control adequately addressed the potential human health and safety concerns associated with NeabNPV and microbial contaminants introduced during production. The acute toxicity studies submitted in support of registration of Abietiv Technical and Abietiv were reviewed. The data set included acceptable acute oral, acute pulmonary, injection, dermal toxicity/irritation and hypersensitivity studies. No overt signs of toxicity were noted when Abietiv Technical was administered to study animals via the oral, pulmonary, intravenous and dermal routes of exposure. Dermal irritation was assessed in the dermal toxicity study. Abietiv was found to be minimally to slightly irritating based on the maximum average score (MAS) of 0.57 and maximum irritation score (MIS) of 0.9 at 24 hours, respectively. An eye irritation study was not submitted and an eye irritation caution is therefore required on product labels.

A tissue culture study was submitted and was considered acceptable but supplemental due to a number of deviations from the guideline requirements. A number of published studies, however, demonstrate that related baculoviruses are not cytopathic to a range of mammalian cells. Based on PMRA's knowledge and familiarity with baculoviruses in general and information in the available published scientific literature, no further tissue culture testing is required to assess infection (overt, persistent, latent or abortive), transformation and toxicity.

With respect to potential effects on the endocrine system, no reports or indications in the available scientific literature suggest that NeabNPV Newfoundland strain in Abietiv has caused or has the potential to cause adverse effects on the endocrine system of animals.

The formulants used in Abietiv are not of toxicological concern.

3.2 Hypersensitivity Incidence

A dermal sensitization study was conducted with a related baculovirus product, Lecontvirus (*N. lecontei* NPV), consisting of lyophilized finely ground redheaded pine sawfly larvae infected with nuclear polyhedrosis virus (2.0×10^{10} PIB/g) in phosphate buffered saline. Eight young adult male guinea pigs were tested and each animal received 0.01 g of test substance in a 0.1 mL intradermal injection on alternate days over a period of three weeks. Animals were challenged with a final injection of the test substance two weeks after the last injection. At 24 and 48 hours after injection, calipers were used to determine double skin thickness at the injection sites as a measure of dermal reactions. Skin lesion diameters were also measured. The same measurements were made for the negative and positive controls. Dermal reactions to Lecontvirus were more severe than those observed in the negative controls. In this study, Lecontvirus was found to be a sensitizing agent. Therefore, the statement "POTENTIAL SENSITIZER" is required on the principal display panel of the labels for Abietiv Technical and Abietiv. Under the "PRECAUTIONS" section on the secondary display panel, the statement "May cause sensitization" must appear. Continued surveillance and reporting of hypersensitivity incidents are required.

3.3 Impact on Human and Animal Health Arising From Exposure to the Active Substance or to Its Impurities

3.3.1 Occupational and Bystander Exposure Assessment

The human health and safety studies reviewed showed that NeabNPV Newfoundland strain and Abietiv are of low toxicity via the oral, pulmonary, intravenous and dermal routes of exposure. Abietiv was found to be minimally to slightly irritating to the skin. An eye irritation study was not submitted. Consequently, the signal words "CAUTION—EYE IRRITANT" are required on the principal display panel of both the Abietiv Technical and Abietiv end-use product labels. As is the case with all microbial pesticides, NeabNPV Newfoundland strain is considered to be a potential sensitizer and will therefore require the signal words "POTENTIAL SENSITIZER" on the principal display panel of each label.

As described in Section 1.3, the proposed use of Abietiv is as a viral insecticide against balsam fir sawfly in forest stands using aerial application equipment. When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of occupational and bystander exposure. The Agency, however, does not expect that occupational exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for NeabNPV Newfoundland strain, and the assumption that precautionary label statements will be followed in the use of Abietiv. The potential for dermal, eye and inhalation exposure for pesticide handlers (i.e., mixer/loaders) exists, with the major source of exposure to workers being generally dermal. To minimize exposure and risk to mixer/loader workers, it is recommended that the label include standard personal protective equipment (including a long-sleeved shirt, long pants, waterproof gloves, eye goggles, shoes and socks) when handling this product. Specific personal protective equipment is not required for aerial applicators.

The label does not allow applications to turf, residential or recreational areas. Because the use sites are limited to forest stands and wood lots, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible to non-existent.

4.0 Residues

4.1 Residue Summary

Abietiv is proposed for use only in forest stands. It is not applied to food or feed crops, hence exposure via this route is unlikely to occur. The acute oral toxicology data submitted by the applicant and the lack of production of known mammalian toxins, in addition to a long history of research, use and safety testing of baculoviruses, indicate that any inadvertent exposure poses minimal risks.

The establishment of a maximum residue limit (MRL) is not required for NeabNPV Newfoundland strain under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as

defined under Division 15, Section B.15.002 of the Food and Drug Regulations, as it is not intended for application to food or feed crops.

Polyhedral inclusion bodies appear to be strongly adsorbed onto soil particles and are not leached extensively from the soil into neighbouring aquatic environments (Jaques 1969). Baculoviruses are not generally recognized as aquatic microorganisms and are not expected to proliferate in aquatic habitats following incidents of direct or indirect exposure. Both percolation through soil and municipal treatment of drinking water reduce the possibility of exposure to NeabNPV Newfoundland strain through drinking water.

5.0 Fate and Behaviour in the Environment

5.1 Summary of Fate and Behaviour in the Terrestrial Environment

As a result of the conclusion that Abietiv would have minimal non-target impacts (see Section 6.0), no additional studies or data are required to complete the environmental risk assessment of the product. The need for data on environmental fate or advanced ecotoxicological data (i.e., Tier II) was not triggered under current requirements for the proposed product, as results of the initial Tier I tests did not meet the adverse effects criteria that would require additional testing.

6.0 Effects on Non-Target Species

See Table I and Table II of Appendix II, for summary tables.

6.1 Summary of Effects on Non-Target Species

Studies and information related to the potential effects of NeabNPV Newfoundland strain or other related baculoviruses on terrestrial and aquatic organisms were reviewed to assess the impact of Abietiv on non-target organisms. Adverse effects are not expected to be observed in birds, wild mammals, earthworms and other soil macroorganisms or vascular plants due to the use of Abietiv. Non-target insect testing indicates that species closely related to the target pest, *Neodiprion abietis*, in the hymenopteran order may be at risk. However, Abietiv will be limited to forestry uses. Spray applications, at the label rate of 10^9 PIBs/ha, introduce relatively little virus into the environment compared to natural baculovirus epizootics in which single late instar larvae can release 10^9 to 10^{10} PIBs (Cory 2003). Therefore, the use of Abietiv will not significantly increase background levels of the virus in the environment and the potential increased risk to non-target terrestrial organisms will be similarly non-significant.

A study submitted on rainbow trout indicated that the nuclear polyhedrosis virus of the redheaded pine sawfly, a related baculovirus, was not toxic to fish. However, deviations from the guideline requirements rendered the study acceptable but supplementary. A study in the published literature demonstrated that *Lymantria dispar* NPV and *Choristoneura fumiferana* NPV, related baculoviruses, had no adverse effects on rainbow trout (Kreutzweiser et al. 1997). A study submitted on *Daphnia magna* indicated that no adverse effects are expected at nominal

concentrations of up to 10^6 PIBs/mL. At the maximum proposed rate of 3×10^9 PIBs/ha, the 15 cm-expected environmental concentration (EEC) in water is 2 PIBs/mL. A data waiver from non-target aquatic non-arthropod invertebrate studies was submitted based on the following rationale:

- the active ingredient is naturally occurring;
- its level in aquatic environments will not significantly increase with the use of Abietiv;
- the host range associated with the active ingredient NeabNPV is limited; and
- there are no reports of adverse effects in aquatic species of non-arthropod invertebrates with the use of related NPVs.

6.2 Integrated Environmental Fate and Toxicology Summary

According to the proposed use pattern, Abietiv is to be applied to forest stands using aerial spray equipment. Consequently, the potential for exposure to terrestrial non-target species is high. However, the risk to non-target terrestrial and aquatic species is expected to be low based on the results of submitted studies and an absence of adverse effects reported in the published scientific literature with other baculoviruses.

NeabNPV Newfoundland strain is naturally occurring. Spray applications, at the label rate introduce relatively little virus into the environment compared to natural baculovirus epizootics. Therefore, the use of Abietiv will not significantly increase background levels of the virus in the environment and the potential increased risk to non-target organisms will be similarly non-significant. Furthermore, the host range of NeabNPV Newfoundland strain is limited to arthropods in the hymenopteran order. Consequently, Abietiv is expected to pose little environmental risk when used in accordance with the proposed label directions.

The formulants in the end-use product do not pose an environmental risk when used at the proposed application rates.

7.0 Efficacy

7.1 Effectiveness

7.1.1 Intended Use

The Canadian Forest Service of Natural Resources Canada has applied for restricted class registration of Abietiv Flowable Biological Insecticide, containing NeabNPV: 4×10^9 polyhedral inclusion bodies (PIBs) per millilitre. Abietiv Flowable Biological Insecticide is proposed for use in forestry (Use-site Category # 4: Forests and Woodlots) to control Balsam fir sawfly larvae. See the following table for detailed intended uses.

Proposed Label Claims of Abietiv Flowable Biological Insecticide

Pest controlled	Balsam fir sawfly (*Neodiprion abietis*)
Use sites	Forestry
Dosages	One billion PIBs of NeabNPV per hectare
Timing of application	Coincide with sufficient foliage development (shoot elongation or leaf expansion) to ensure maximum spray deposit and targeting complete egg hatch.
Re-application interval	One application per year
Application methods	Aerial application

7.1.2 Mode of Action

The active ingredient of Abietiv Flowable Biological Insecticide is *Neodiprion abietis* nucleopolyhedrovirus, which is a NPV naturally infecting larvae of balsam fir sawfly (*Neodiprion abietis*). NPVs belong to the Baculoviridae family, commonly called baculoviruses. Baculoviruses only infect the larval feeding stage where they form occlusion bodies (or polyhedral inclusion bodies—PIBs for NPVs). These proteinaceous structures contain the virions (virus particles). Histopathological examination of larvae of several sawfly species has revealed that replication of NPV only occurs in midgut epithelial cells. Larval feeding declines and ceases long before death. Transmission of NeabNPV is by either horizontal spread or vertical passage (between generations through adult-to-progeny spread or to subsequent generations by persistence in the environment). NeabNPV is considered species specific, because it has a narrow spectrum of activity.

7.1.3 Use Sites

Abietiv Flowable Biological Insecticide is proposed for forestry use.

7.1.4 Effectiveness Against Pest

Three field efficacy trials (Ontario, Newfoundland) were submitted to support the proposed label claims of Abietiv Flowable Biological Insecticide. The results of these trials demonstrated the following:

1. Abietiv Flowable Biological Insecticide could significantly increase NeabNPV infection rate in the field population of balsam fir sawfly;
2. Abietiv Flowable Biological Insecticide could cause larval mortality at a significant level; and

3. under suitable conditions (i.e., depending on the developmental phase of natural populations and timing of treatment), Abietiv Flowable Biological Insecticide could reduce balsam fir sawfly populations to tolerable levels by causing high mortality in both current treatment year and during the subsequent years.

Application of Abietiv Flowable Biological Insecticide has proven to be a valuable augmentative biological control measure (see Section 7.5.2)

7.1.5 Total Spray Volume

Abietiv Flowable Biological Insecticide is to be diluted in a 20% molasses mixture. For preparation of spray solution, add between 1 and 3 mL of Abietiv Flowable Biological Insecticide in each 10 L of molasses solution. The higher rates are to be used under higher pest pressure. The spray solution will yield a rate of 1 to 3 billion (10^9) PIBs of NeabNPV, when 2.5 L of the spray mixture is applied per hectare.

7.2 Phytotoxicity to Target Plants or Target Plant Products

No specific testing of the effects of NeabNPV on plants was submitted, and no phytotoxicity studies have been requested by the PMRA. NeabNPV is a naturally occurring organism, known for its selectivity against balsam fir sawfly. No adverse effects on flora or non-target fauna are expected.

7.3 Impact on Succeeding Crops, Adjacent Crops and on Treated Plants or Plant Products Used for Propagating

N/A

7.3.1 Impact on Adjacent Crops

No adverse effects on flora or non-target fauna are expected where NeabNPV is applied to reduce populations of balsam fir sawfly.

7.3.2 Tank Mixing Recommendations

Abietiv Flowable Biological Insecticide is proposed for use alone and not for tank mixing with other products.

7.4 Economics

Not assessed.

7.5 Sustainability

7.5.1 Survey of Alternatives

No alternative products are registered for the control of balsam fir sawfly in forestry settings.

7.5.2 Compatibility with Current Management Practices Including Integrated Pest Management

Application of Abietiv Flowable Biological Insecticide is an augmentative biological control measure, because NeabNPV naturally exists in the field and plays a significant role in natural population dynamics. Due to its narrow spectrum of activity, Abietiv Flowable Biological Insecticide has added value as an IPM compatible (species specific) pest control product.

7.6 Conclusions

Based on the submitted data and information, registration of the proposed product can be supported from a value point of view for use in forestry to reduce populations of balsam fir sawfly at the proposed application rate (10^9 PIBs/ha). In addition, higher rates (up to 3×10^9 PIBs/ha) are recommended for use under higher pest pressure, which is based on the dose-response data and actual field testing data at 3×10^9 PIBs/ha.

7.6.1 Summary

Summary of Label Proposals and Recommendations of Abietiv Flowable Biological Insecticide

Proposed Use Claims	Accepted Use Claims
Site/Pest: For use in forestry for control of balsam fir sawfly larvae (*Neodiprion abietis*)	**Site/Pest:** For use in forestry to reduce populations of balsam fir sawfly (*Neodiprion abietis*)
Application: One aerial application per year	**Application**: One aerial application per year
Timing: Coincide with foliage development (shoot elongation or leaf expansion) to insure maximum spray deposit and targeting at complete egg hatch	**Timing:** Coincide with foliage development (shoot elongation or leaf expansion) to ensure maximum spray deposit and targeting at complete egg hatch
Application rate: 1×10^9 PIBs/ha	**Application rate:** 1×10^9 to 3×10^9 PIBs/ha

8.0 Toxic Substances Management Policy Considerations

During the review of Abietiv, the PMRA has taken into account the federal Toxic Substances Management Policy[1] and has followed its Regulatory Directive DIR99-03[2]. It has been determined that this product does not meet TSMP Track 1 criteria, because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. Furthermore, the microbial pest control agent does not contain any byproducts or microcontaminants that meet the TSMP Track 1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated in sufficient quantities during the manufacturing process to present a risk to human health and safety. Also, there are no formulants of toxicological concern present in Abietiv.

9.0 Regulatory Decision

9.1 Regulatory Decision

The active ingredient NeabNPV Newfoundland strain and associated end-use product, Abietiv, have been granted temporary registration to reduce populations of balsam fir sawfly in forest stands, pursuant the Pest Control Product Regulations, subject to the following data requirements outlined in Section 9.2.

9.2 Additional Data Requirements

Storage stability data are required. A range of storage temperatures and time periods are recommended.

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1 The federal Toxic Substances Management Policy is available through Environment Canada's website at www.ec.gc.ca/toxics.

2 Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*, is available through the Pest Management Information Service. Phone: 1-800-267-6315 within Canada or 613-736-3799 outside Canada (long distance charges apply); Fax: 613-736-3798; e-mail: pmra_infoserv@hc-sc.gc.ca; or through our website at www.pmra-arla.gc.ca.

List of Abbreviations

Bt	*Bacillus thuringiensis*
EEC	expected environmental concentration
ha	hectare
IPM	integrated pest management
LD_{50}	lethal dose to 50% of a population
LD_{95}	lethal dose to 95% of a population
LOEC	lowest observed effect concentration
MAS	maximum average score
MIS	maximum irritation score
MRL	maximum residue limit
NeabNPV	*Neodiprion abietis* nucleopolyhedrovirus
NPV	nucleopolyhedrovirus
OECD	Organisation for Economic Co-operation and Development
PIB	polyhedral inclusion body
PMRA	Pest Management Regulatory Agency
TSMP	Toxic Substances Management Policy

Appendix I Toxicology

Table 1 Toxicology Table

STUDY	SPECIES, STRAIN AND DOSES	RESULT	TARGET ORGAN, SIGNIFICANT EFFECTS, COMMENTS
ACUTE STUDIES			
Oral toxicity	Rat—Sprague-Dawley CD 7.5×10^7 PIBs/animal	LD_{50}, $> 7.5 \times 10^7$ PIBs/animal	No mortalities and no clinical signs of toxicity. No gross necropsy findings. **LOW TOXICITY**
Pulmonary toxicity	Rat—Sprague-Dawley CD 9.0×10^7 PIBs/animal	$LC_{50} > 9.0 \times 10^7$ PIBs/animal	No mortalities and no clinical signs of toxicity. No gross necropsy findings. **LOW TOXICITY**
Injection (intravenous)	Rat—Sprague-Dawley CD, 10^7 PIBs/animal	Not toxic	No mortalities and no clinical signs of toxicity. No gross necropsy findings. **NOT TOXIC**
Dermal toxicity	Rabbit—New Zealand white 4.0×10^9 PIBs/mL	LD_{50}, $> 4.0 \times 10^9$ PIBs/mL	No mortalities and no clinical signs of toxicity. **LOW TOXICITY**
Dermal irritation	Rabbit—New Zealand white 4.0×10^9 PIBs/mL	MIS = 0.9 on day 1 MAS = 0.57	On day 1, 3 males and 4 females exhibited very slight erythema and 1 female exhibited well-defined erythema. One male continued to have slight erythema from days 2 to 6. Two females had slight erythema on day 2. All animals returned to normal by day 9. There was no edema. **MINIMALLY TO SLIGHTLY IRRITATING**
Hypersensitivity	Guinea pig 2.0×10^8 PIBs/mL	Potential sensitizer	Label statement requirement: **"POTENTIAL SENSITIZER"**
Tissue culture	Published literature	Not infective	None
Eye irritation	Not submitted		Label statement requirement: **"CAUTION—EYE IRRITANT"**

Appendix II Environmental Assessment

Table 1 Risks of Abietiv to Non-Target Terrestrial Organisms

Organism	Exposure	Test Substance	Conclusions
Arthropods	Acute	• Study summaries on non-target terrestrial arthropods (*Acantholyda erythrocephala, Diprion similis, Gilpinia hercyniae, Pristiphora geniculata, Choristoneura rosaceana, Clepsis persicana, Melanchra pulverulenta, Pyrrhia exprimens*) • Published literature on related baculoviruses • Waiver rationale	The study summaries demonstrated a limited host range for NeabNPV Newfoundland strain. Species closely related to the target pest, *Neodiprion abietis*, in the hymneopteran order may be at risk. However, the published literature and waiver rationale were **ACCEPTED** and indicated a limited potential for risk.
Birds	Oral/ Pulmonary/ Inhalation/ Injection	Study on Hubbard White Mountain broiler chickens and White Hybrid turkeys using the nuclear polyhedrosis virus of the redheaded pine sawfly	No mortalities and no treatment-related clinical signs or findings at necropsy. These studies using redheaded pine sawfly nuclear polyhedrosis virus can be bridged to Abietiv due to the close taxonomic relationship between these two species of baculoviruses. **LOW TOXICITY ACCEPTABLE**
Wild mammals	Acute	Waiver rationale submitted in lieu of data.	The waiver rationale submitted by the applicant was **ACCEPTED** based on a lack of significant adverse effects in the published literature and in the human health and safety studies.
Plants	Acute	• Observations made during efficacy trials • Published literature • Waiver rationale	No adverse effects on terrestrial plants were observed during efficacy trials. The published literature and waiver rationale were **ACCEPTED** based on a limited potential for risk.
Soil microorganisms	Acute	Not required	This data requirement was not triggered.
Non-arthropod invertebrates	Acute	Waiver rationale submitted in lieu of data.	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.

Table 2 Risks of Abietiv to Non-Target Aquatic Organisms

Organism	Exposure	Test Substance	Conclusions
Freshwater arthropods	Acute	• Study on *Daphnia magna*	No adverse effects on parental mortality or fecundity were observed. The LOEC and 21-day LC50 were determined to be > 10^6 PIBs/mL. The NOEC value, based on mortality/sublethal effects was found to be 10^6 PIBs/mL. **LOW TOXICITY ACCEPTABLE**
Freshwater fish	Acute	• Study on rainbow trout using the nuclear polyhedrosis virus of the reheaded pine sawfly • Published literature	The 30-day LD_{50} in the acute oral toxicity and the 30-day LC_{50} in the contaminated water tests were > 3×10^6 PIBs/g bw and > 2.4×10^4 PIBs/mL, respectively. The NOEL value, was > 3×10^6 PIBs/g bw. The EC_{50} and NOEC values, based on mortality/sublethal effects, were both > 2.4×10^4 PIBs/mL. Sublethal effects consisting of kidney, gill and stomach abnormalities were observed in the groups exposed to the test substances and in the controls. There were no mortalities. Based on the results of this study, *Neodiprion lecontei* nucelopolyhedrosis virus is of **LOW TOXICITY** to rainbow trout. This study was classified as **ACCEPTABLE** but supplementary due to deviations from the guideline requirements (e.g., size of fish, low dose in the water contamination study, viability of test substance was not assessed). The published literature was **ACCEPTED** based on a limited potential for risk.
Aquatic non-arthropod invertebrates	Acute	Waiver rationale submitted in lieu of data.	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.
Freshwater plants	Acute	Not required	This data requirement was not triggered.

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